Morphology and Cytochemistry of Bovine Bone Marrow Mononuclear Phagocytes

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ABSTRACT

Bone marrow samples were collected from five normal calves and mononuclear cells were separated using Ficoll-Hypaque. Mononuclear cells were cultured on coverslips in Leighton tubes for six hours. The adherent cells were differentiated using Wright's and nonspecific esterase stains. Monoblasts, promonocytes and monocytes were present in the proportion of 1:2.31:4.96.

RÉSUMÉ

Cette expérience consistait à prélever des échantillons de moelle osseuse, chez six veaux normaux, et à en récolter les mononucléaires, à l'aide du mélange Ficoll-Hypaque. On cultiva ensuite ces mononucléaires, sur des lamelles, dans des tubes de Leighton, pour une période de six heures. On différencia les cellules qui avaient adhéré aux lamelles, à l'aide de la coloration de Wright et de celle de l'estérase non spécifique. On constata ainsi la présence de monoblastes, de promonocytes et de monocytes, dans les proportions suivantes: 1: 2,31: 4,96.

INTRODUCTION

Bone marrow mononuclear phagocytes constitute a distinct cell line and arise from an immature cell termed the "monoblast." A monoblast divides once, giving a rise to two promonocytes which, in their turn, divide once and each form two nonproliferating monocytes (4). Monocytes leave the bone marrow and are transported via the blood to various tissues where they mature to become macrophages (6). Identification of monoblasts and promonocytes in bone marrow cytological preparations has been difficult because of the variety of immature and mature cell types as well as the low numbers of monoblasts and promonocytes, i.e. about 0.25% promonocytes in bone marrow (7). Based on the property of mononuclear phagocytes to adhere to glass surfaces (6), promonocytes and monocytes have been differentiated and characterized morphologically and cytochemically in bone marrow cultures from mice (7). However, monoblasts were not observed in these cultures. In the present study, a bone marrow adherent cell culture system was employed to isolate, differentiate and characterize bovine bone marrow mononuclear phagocytes morphologically and cytochemically.

MATERIALS AND METHODS

ANIMALS

Five three month old castrated male Holstein-Friesian calves were used as marrow donors. Their weights varied from 77 to 86 kg. Clinical and hematological examinations were performed prior to the experiment to ensure that all animals were clinically normal. The jugular area on both sides of the neck and the sternal region were clipped, washed and disinfected with 95% ethanol.

MARROW COLLECTION

The calves were restrained in lateral recumbency following an intravenous injection of xylazine[1] at a dosage of 0.25 mg/kg body weight. Their sternal regions were disinfected with 95% ethanol, and the marrow cavity of the third or fourth sternebra was penetrated with a 16 gauge four cm marrow biopsy needle.[2] Three to 5 mL of marrow were aspirated into a plastic disposable 12 mL syringe.[3] The sample was quickly transferred into a 17 x 100 mm (15 mL) polystyrene disposable test tube[4] containing 100 units of heparin[5] and 3 mL of RPMI 1640 medium.[6] The marrow sample was diluted 1:1 by adding more RPMI medium.

BONE MARROW CELL CULTURES

The mononuclear cells of the

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[1]Rompun, Haver-Lockhart Laboratories, Division of Bayvet Corporation, Mississauga, Ontario, Canada.
[2,3]Arnold-Nasco Limited, Guelph, Ontario, Canada.
[4]Fisher Scientific Co. Ltd., Whitby, Ontario, Canada.
[5]Allen and Hanburys, Toronto, Ontario, Canada.
[6,9]Grand Island Biological Co., Burlington, Ontario, Canada.

bone marrow were separated as described by Walker *et al* (8), except that Ficoll[7]-Hypaque[8] was used instead of a Ficoll-Isopaque mixture. Three cultures from each of five calves were prepared by seeding 4 x 10^6 nucleated marrow cells in 2 mL of RPMI 1640 medium containing 100 units penicillin/mL, 50 μg streptomycin/mL and 20% fetal calf serum.[9] Two mL of cell suspension were transferred into a screw-capped Leighton tube[10] (16 x 93 mm) containing a 10.5 x 35 mm coverslip.[11] All cultures were incubated for six hours in an atmosphere containing 5% CO_2 and high humidity, and at a temperature of 37°C. At the end of the incubation time, the cultures were washed three times with RPMI 1640 medium and air dried. One culture from each calf was stained for nonspecific esterase activity (9) and another was stained with Wright's stain. One hundred nucleated cells and 100 mononuclear phagocytes were differentiated from each culture. One culture from each calf was stained for peroxidase activity according to Yam *et al* (9), except that the incubation period used was two minutes instead of 30 seconds.

RESULTS

The cells were differentiated in Wright's and nonspecific esterase stained bone marrow cultures. The differential counts from these cultures indicated that 59.4% of the adherent cells were mononuclear phagocytes (Table I). The mononuclear phagocytes were further differentiated into monoblasts, promonocytes and monocytes according to their morphological and cytochemical characteristics (Table II). The monoblast was round and had a round nucleus surrounded by a small rim of basophilic cytoplasm (Fig. 1a). The nucleus was the most basophilic of the three cell stages, the chromatin was aggregated, particularly around nucleoli, and usually had numerous small unstained circular areas. The nuclear to cytoplasmic ratio was greater than one. The promonocyte had an indented or folded nucleus and usually contained one nucleolus (Fig. 1b). There was more prominent clumping of chromatin with a diffuse chromatin distribution between clumps. The cytoplasm was less basophilic than that of a monoblast and a distinct perinuclear clear area was often observed. The nuclear to cytoplasmic ratio was greater than one but less than that of the monoblast. The monocyte usually had a horeshoe shaped nucleus (Fig. 1c). The nucleus was least basophilic and there was less clumping of chromatin with predominance of a diffuse chromatin pattern. The cytoplasm was grayish blue, and contained vacuoles and granules. The nuclear to cytoplasmic ratio was one or slightly less.

The bone marrow mononuclear phagocytes were positive for non-

TABLE I. Percentage of Cells in Six Hour Bone Marrow Cultures From Five Calves as Determined by Two Different Staining Techniques

	Type of Cell		
Type of Stain	Granulocytes %	Mononuclear Phagocytes %	Lymphocytes %
Wright's stain	40 ± 8[a]	58 ± 7.3	2 ± 1.4
Nonspecific esterase	37.8 ± 5.1	59.4 ± 5.7	2.8 ± 1.5

[a]Mean ± Standard Deviation

TABLE II. The Composition of Mononuclear Phagocytes in Six Hour Bone Marrow Cultures From Five Calves According to Two Different Staining Techniques

	Type of Cell		
Type of Stain	Monoblasts %	Promonocytes %	Monocytes %
Wright's stain	11.5 ± 2.6[a]	28.6 ± 2.1	59.9 ± 4.1
Nonspecific esterase	12.1 ± 0.7	27.9 ± 1.1	60 ± 2.4

[a]Mean ± Standard Deviation

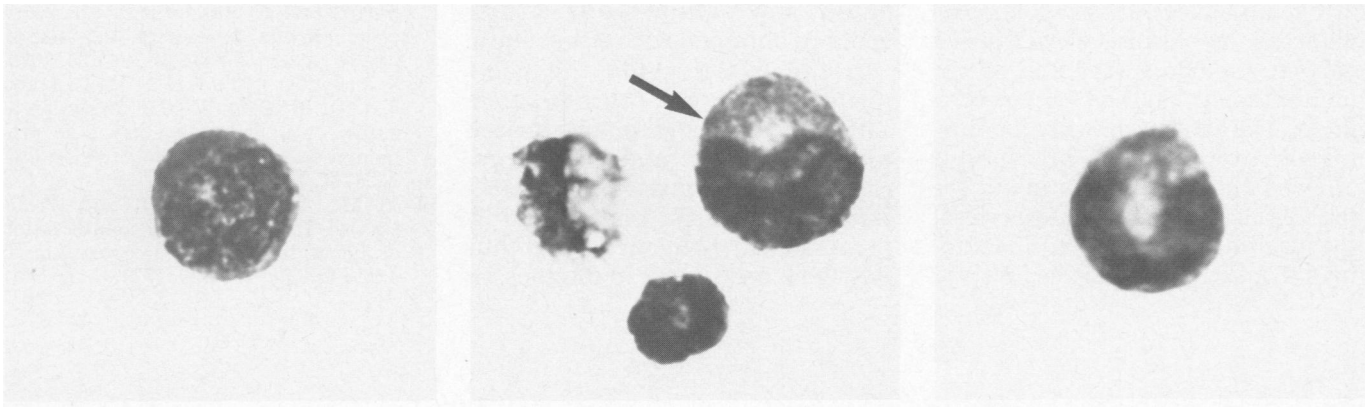

Fig. 1. Monoblast (a), promonocyte (b, arrow) and monocyte (c) in six hour bovine bone marrow culture. Wright's stain. X1250.

[7]Pharmacia Fine Chemicals, Uppsala, Sweden.
[8]Winthrop Laboratories, Aurora, Ontario, Canada.
[10,11]Bellco Glass, Inc., Vineland, New Jersey, U.S.A.

specific esterase activity and the cytoplasmic staining increased in intensity from monoblast to monocyte. The ratio between monoblasts, promonocytes and monocytes was 1:2.49:5.21 in Wright's stained cultures and 1:2.31:4.96 in nonspecific esterase stained cultures. Weak peroxidase activity was observed in the cytoplasm of monoblasts and monocytes, whereas promonocytes reacted moderately for peroxidase activity.

The adherent granulocytes were metamyelocytes, bands and segmented granulocytes. These cells were negative for nonspecific esterase activity and strongly positive for peroxidase activity. The lymphocytes do not have the ability to adhere to glass surfaces (2), and most of them were detached during the washing process.

DISCUSSION

In the present study, three types of mononuclear phagocytes, monoblasts, promonocytes and monocytes, were identified and differentiated according to their morphological and cytochemical characteristics in six hour bone marrow cultures. These results expand upon those of Van Furth and Cohn (6) and Van Furth *et al* (7) in which monoblasts were not noticed in the cultures but their techniques are slightly different from those used in our study. These authors washed the cultured cells at two and six hours of incubation whereas the cultures were washed only at six hours (the end of the incubation period) in our experiment. The absence of washing during the first six hours might have allowed the monoblasts to adhere to the glass surface and thus resist the washing by six hours. The findings from our study agree, in part, with that of Mori *et al* (5) who grew macrophage colonies from adherent cells indicating that colony forming cells (monoblasts) were present in these cultures. Their results indicated that the monoblasts had the ability to adhere to glass surfaces if the cultures were not disturbed for one day or more.

The ratio between monoblast, promonocyte and monocyte (1:2.31:4.96) supported the conclusion that a monoblast divides once, giving rise to two promonocytes which, in turn, divide once and each form two nonproliferating monocytes as demonstrated in mice (4).

The morphological features of mononuclear phagocytes in six hour cultures indicate that the monoblast is the most immature cell, the promonocyte is an intermediate cell and the monocyte is the most mature cell. A similar classification has been reported in mice (3). Monoblasts, promonocytes and monocytes are strongly positive for nonspecific esterase activity and weakly positive for peroxidase activity except for promonocytes which are moderately positive. Cytochemically, these cells are similar to those of the mouse (1, 3). Identification of the various stages of mononuclear phagocytes was much easier and more precise in six hour bone marrow cultures than in direct cytological preparations. The differential counts in nonspecific esterase and Wright's stained cultures indicated that comparable results could be obtained with either stain.

In the present study, the mononuclear phagocytes were isolated, differentiated and characterized morphologically and cytochemically. This information can be utilized to identify bone marrow mononuclear phagocytes and thus may be a useful tool in diagnosing some diseases such as monocytic leukemia.

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